

REVIEW



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Alcohol-associated capillarization of sinusoids: A critique since the discovery by Schaffner and Popper in 1963

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Abstract

This article reviews the literature on capillarization of hepatic sinusoids since its discovery in 1963. Liver sinusoidal endothelial cells are uniquely fenestrated and lack an underlying basement membrane. In chronic liver disease, the sinusoids capillarize and transform into systemic capillaries, a process termed capillarization of sinusoids. The histopathology is marked by defenestration, basement membrane formation, and space of Disse fibrogenesis. Capillarized sinusoids compromise the bidirectional exchange of materials between sinusoids and hepatocytes, leading to hepatocellular dysfunction. Sinusoidal capillarization was first described in active cirrhosis of alcoholics in 1963. Since then, it has been found in early and progressive stages of alcoholic hepatic fibrosis before the onset of cirrhosis. The sinusoidal structure is not altered in alcoholic steatosis without fibrosis. Defenestration impairs the ability of the endothelium to filter chylomicron remnants from sinusoids into the Disse's space, contributing to alcohol-induced postprandial hyperlipidemia and possibly atherosclerosis. Ethanol also modulates the fenestration dynamics in animals. In baboons, chronic alcohol consumption diminishes endothelial porosity in concomitance with hepatic fibrogenesis and in rats defenestrates the endothelium in the absence of fibrosis, and sometimes capillarizes the sinusoids. Acute ethanol ingestion enlarges fenestrations in rats and contracts fenestrations in rabbits. In sinusoidal endothelial cell culture, ethanol elicits fenestration dilation, which is likely related to its interaction with fenestration-associated cytoskeleton. Ethanol potentiates sinusoidal injury caused by cocaine, acetaminophen or lipopolysaccharide in mice and rats. Understanding ethanol's mechanisms on pathogenesis of sinusoidal capillarization and fenestration dynamics will lead to development of methods to prevent risks for atherosclerosis in alcoholism.

KEYWORDS

alcoholic liver disease, capillarization of sinusoids, defenestration, endothelial fenestrations, ethanol feeding

All authors are contributed equally to this study.

1 | INTRODUCTION

Fenestrations of liver sinusoidal endothelial cells (LSECs) are dynamic structures. Agents such as ethanol, drugs, toxins, growth factors, hormones, viruses, and nutrition have been demonstrated to modulate the dynamics of endothelial fenestrations and impact capillarization of sinusoids. Chronic liver diseases like alcoholic liver disease, nonalcoholic fatty liver disease, and chronic hepatitis viral infection are known to provoke sinusoidal capillarization. Since the discovery of the capillarization of sinusoids in cirrhosis of alcoholics by Schaffner and Popper in 1963, alcoholic liver disease and ethanol feeding in experimental animals represent the single most

engaging field of investigations into the pathogenesis of capillarization of sinusoids. Since the literature lacks a review on alcohol-associated sinusoidal capillarization and defenestration, this article presents an overview of the published studies in the past 58 years. The review begins with an introduction to the terminology related to endothelial fenestration morphology followed by a description of the ultrastructural diagnostic features of capillarization of sinusoids. Next, we categorize the various publications under the headings of alcoholic liver disease, chronic ethanol feeding and acute ethanol ingestion in animals, in vitro effects of ethanol in LSEC culture, and ethanol and hepatotoxic drugs combined. The incidence of capillarization of sinusoids and changes in

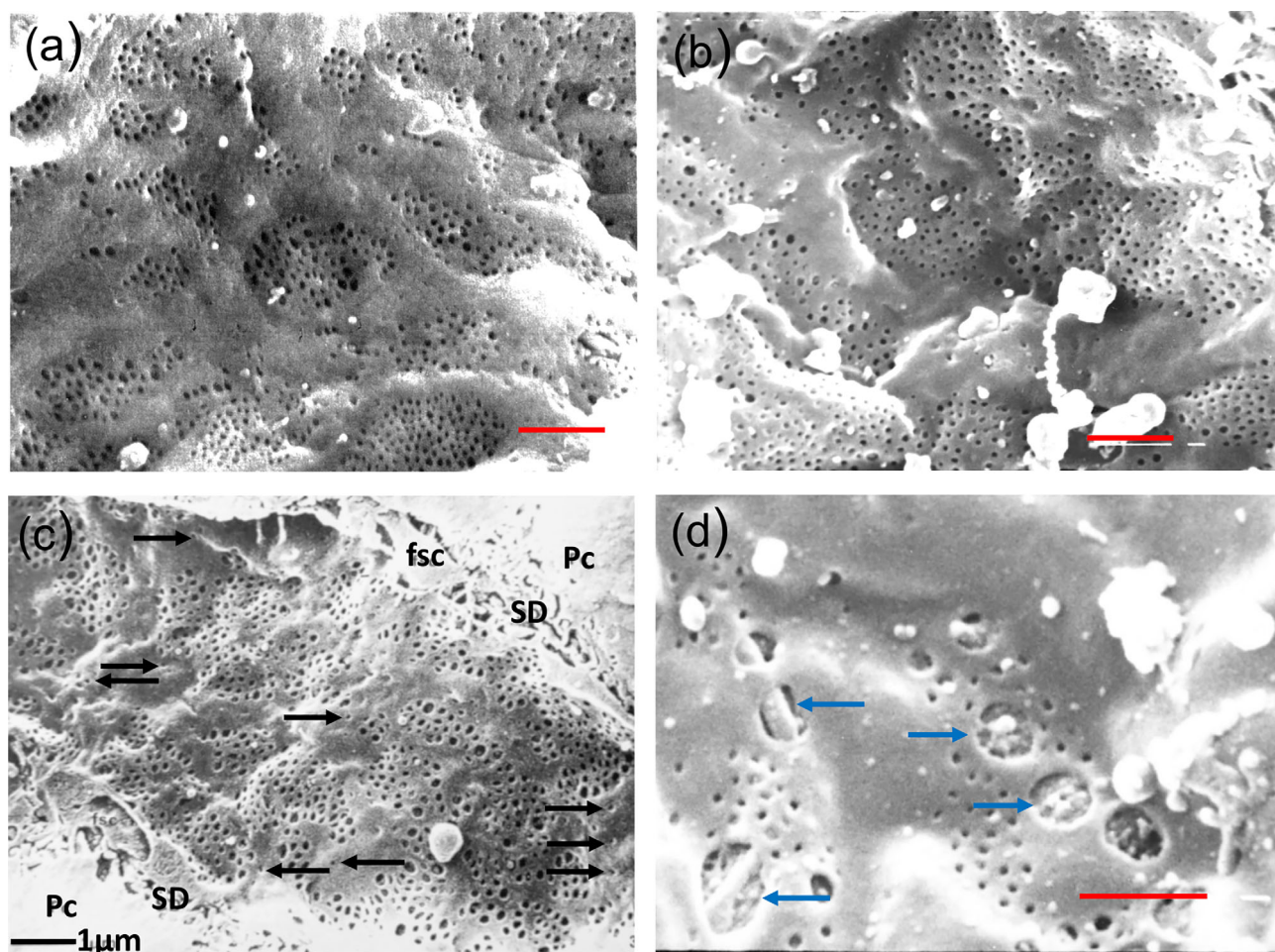


FIGURE 1 SEM micrographs showing a luminal view of sinusoids of human (a), baboon (b), and rat (c). In all species, the fenestrations of endothelial cells are clustered into sieve plates that are separated by nonfenestrated cytoplasmic ridges. In (c), arrows mark cell surface pits. Pc, parenchymal cell; sp, space of Disse; fsc, fat storing cell (hepatic stellate cell). (d) This image was taken from an alcohol-fed baboon to illustrate gap formation. Here, the gaps are in juxtaposition with the fenestrated sieve plates—for illustration, four of them are indicated with arrows. The gaps are $\sim 300 \mu\text{m}$ in diameter or greater, whereas the fenestrations are smaller. The presence of gaps within the sieve plates suggests that they are formed by the fusion of the fenestrations. Through the gaps, one can observe part of an HSC or microvilli in the underlying space of Disse. Scale one bars = $1 \mu\text{m}$. (a) Motta et al., Plate 5.8. The liver: an atlas of Scanning Electron Microscopy, 1978, with permission of Igaku-Shoin Medical Publishers Ltd., Tokyo; Japan. (b) Mak and Lieber (1984), with permission of Wiley. (c) Wisse et al. (1985), with permission of Wiley

fenestration number, diameter, and porosity are tabulated, which provide a platform for discussion of the concordance and discordance of the published findings. Finally, we examine the notion of “sieve plate and hyperlipoproteinemia and atherosclerosis” in alcoholism that implicates a link between defenestration and diminished sieving of chylomicron remnants from the sinusoidal circulation into the space of Disse. The consequential hyperlipidemia may promote the pathogenesis of systemic vascular disease in association with alcohol abuse.

2 | TERMINOLOGY

Fenestrations (or *fenestrae*) are circular or polygonal pores that perforate the sinusoidal endothelial cell cytoplasm (Figures 1 and 2). Their sizes range from 50 to 300 nm in

diameter, depending on the animal or human species derived from, age, and methods of visualization by microscopy. There are 3–20 fenestrations per μm^2 of the endothelial surface area, which cover as much as 20% of the cell surface (Braet & Wisse, 2002; Cogger et al., 2020; Fraser et al., 2012; Mak & Shin, 2021; Sorensen & Smedsrod, 2020; Wisse et al., 1999). The mean lifespan of mouse fenestrations is ~ 18 hr (Zapotoczny et al., 2019). Fenestrations of the hepatic sinusoid lack both bridging diaphragms and underlying basal lamina and their sizes and numbers are heterogenous across the liver lobule. In rats, periportal fenestrations are larger in diameter and fewer in number whereas centrilobular fenestrations are smaller in diameter and greater in number, which was first observed and reported by Wisse et al. (1983) and later in other studies (Kanaoka et al., 1988; Okanoue et al., 1999; Takashimizu et al., 1999). For fine structure

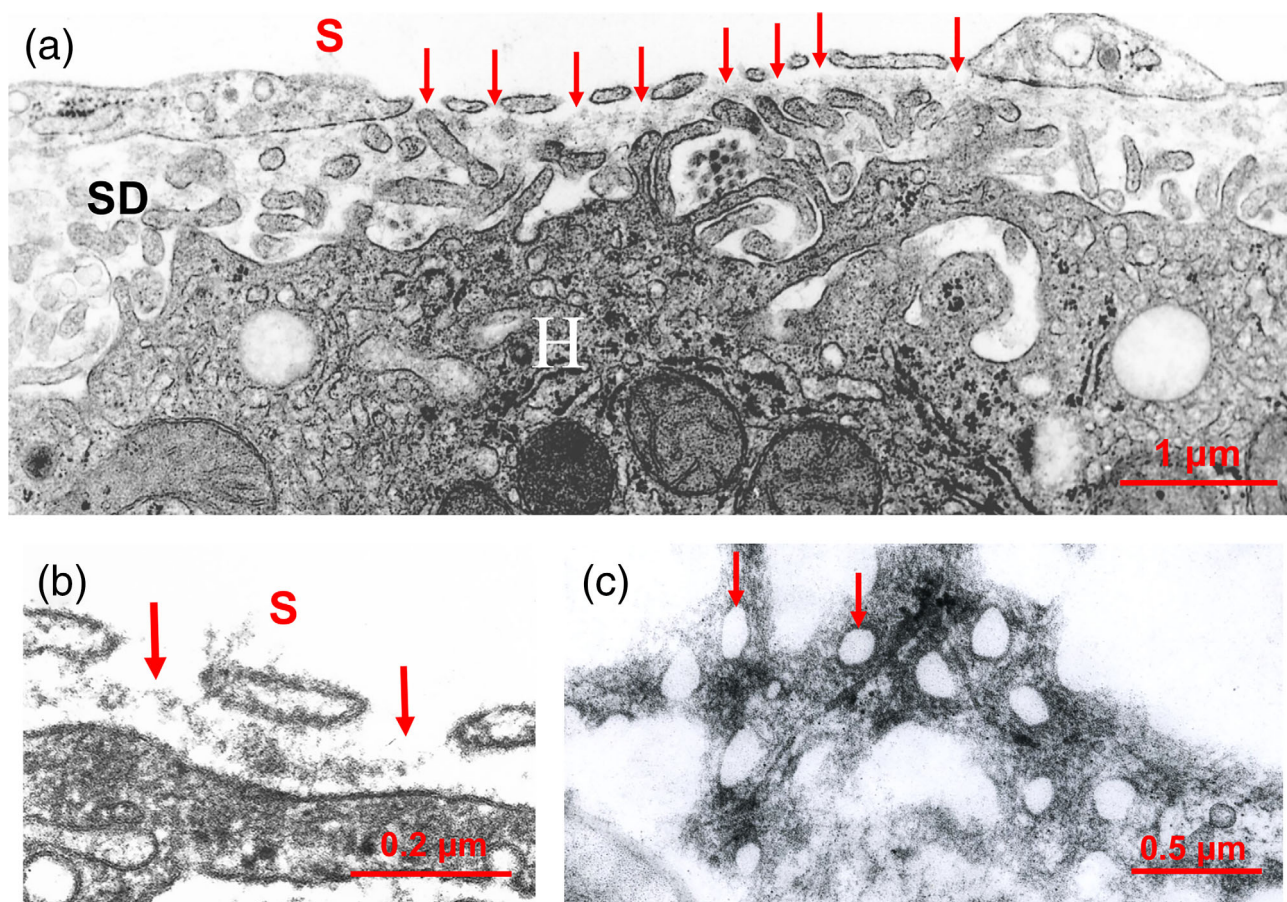


FIGURE 2 TEM micrographs of endothelial fenestrations. (a) This image was taken to show fenestrations in the endothelial cytoplasmic process and the underlying space of Disse. The cytoplasm is thinned out and is perforated by fenestrations about 100–200 nm wide (arrows). Some filamentous material is present in the cytoplasm. The thickened portion of the cytoplasmic process is nonfenestrated and contains few profiles of organelles. The endothelium lacks an underlying basal lamina. The space of Disse is narrow, 0.5–1.5 μm wide, containing fibrillar matrix. The hepatocyte (H) projects short microvilli into the space of Disse, enhancing the surface of area and promoting exchanges of metabolites. (b) Higher magnification of endothelial fenestrations (arrows), measuring 100–110 nm in width. Note absence of diaphragms bridging the fenestrations. (c) Tangential view of endothelial fenestrations: some appear circular and some ovoid (arrows for example). S, sinusoid; SD, space of Disse. (a) Mak and Lieber (1984) with permission of Wiley

of endothelial cells and their immunocytochemical markers, see reviews by Wisse et al. (1999) and Mak and Shin (2021).

A *sieve plate* contains fenestrations clustered into groups of tens to hundreds (Wisse, 1970; Wisse et al., 1985). Sieve plates are found predominantly in the attenuated cytoplasm of the endothelial cell. Between 60% to 75% of fenestrations are found within the sieve plates with the other fenestrations scattered individually across the endothelial cell surface. The number of sieve plates appears to be greater in the centrilobular zone than in the periportal zone (Vidal-Vanaclocha & Barberá-Guillem, 1985). The sieve plate is the major site that regulates filtration/sieving of fluid, solutes, particles, and metabolites from the sinusoid blood into the space of Disse and thence to hepatocytes.

Porosity of the endothelial cell is defined as the percent of surface area covered with fenestrations. Calculation of fenestration porosity takes into account the fenestration diameter (size) as well as the number (frequency). Thus, the porosity of the sinusoidal endothelium expresses the capacity for material transfer from the sinusoid blood circulation into the space of Disse. Porosity displays a periportal to centrilobular gradient. In rats, periportal porosity (6%) is lower than centrilobular porosity (8%) (Wisse et al., 1983). Similarly, in humans, periportal porosity is 7.6% whereas centrilobular porosity is 9.3% (Horn et al., 1987).

Defenestration is a process of reduction in the number or diameter of the fenestrations.

Gaps are large pores greater than 300 nm in diameter—the largest of the fenestrations—and exceed 1 μm . Gap formation reflects destabilization of the fenestrations that results in a coalescence of 2 or more small-sized fenestrations (Wisse et al., 1985). Gaps can occur as a result of cellular injury, high pressure perfusion either physiologically or experimentally, hypoxia, tissue fixation procedures, or pathological states (Cogger et al., 2020). In some investigations gaps were excluded from calculations of the extent of endothelial porosity (O'Reilly et al., 2010).

Basal lamina represents the lamina densa of the three-layered components of the basement membrane, which is observed by TEM, while basement membrane per se is the structure visible by light microscopy (Mak & Mei, 2017).

2.1 | General comments

The morphology of endothelial fenestrations and the dynamics of associated cytoskeleton have been visualized and quantified by various microscopic techniques,

which was first scanning electron microscopy (SEM) and transmission EM (TEM), followed by high-resolution correlative imaging microscopy, atomic force microscopy, structured illumination microscopy, and direct stochastic optical reconstruction microscopy (Braet, 2004; Braet et al., 1996; Braet et al., 2007; Cogger et al., 2010; Cogger et al., 2020; Monkemoller et al., 2014; Zapotoczny et al., 2019). However, the data on fenestration sizes generated from these various methodologies appear to vary. Moreover, aside from SEM and TEM, these other methods have not been widely employed by standard microscopy laboratories to evaluate changes of fenestrations in response to experimental conditions. Yet, when comparing SEM and TEM measurements, SEM measurements of fenestration size tend to be 20–30% smaller as compared to TEM, attributable to the shrinkage of 30% of tissue during SEM processing (Braet et al., 1996; Cogger et al., 2020; Wisse et al., 1985). In the reports included in this review, the fenestration diameters were determined by SEM.

3 | CAPILLARIZATION OF HEPATIC SINUSOIDS: A TRIPARTITE STRUCTURAL CHANGE ENCOMPASSING ENDOTHELIAL DEFENESTRATION, PERISINUSOIDAL BASEMENT MEMBRANE FORMATION, AND PERISINUSOIDAL FIBROSIS OF THE SPACE OF DISSE

Capillarization of sinusoids is a significant pathology associated with chronic liver disease and encompasses thickening of the endothelium with loss of endothelial fenestrations (defenestration), deposition of a basal lamina beneath endothelial cells, and ultimately phenotypic transformation of the sinusoidal endothelium into a systemic vascular-type capillary (Figure 3). These structural changes were termed capillarization of hepatic sinusoids by Schaffner and Popper in 1963. The histogenesis of capillarized sinusoids along with increased deposition of collagen types I, III, V, VI, and XVIII (Mak & Mei, 2017) and matrix molecules such as fibronectin and proteoglycans have been found to be associated with a widened space of Disse: from a normal width of 0.5 μm to several μm in many instances—namely perisinusoidal fibrosis. In addition, the hepatocyte microvilli facing the space were found to be denuded. It is worth noting that the sinusoidal endothelium may defenestrate even before the appearance of a basement membrane. Sinusoidal capillarization can

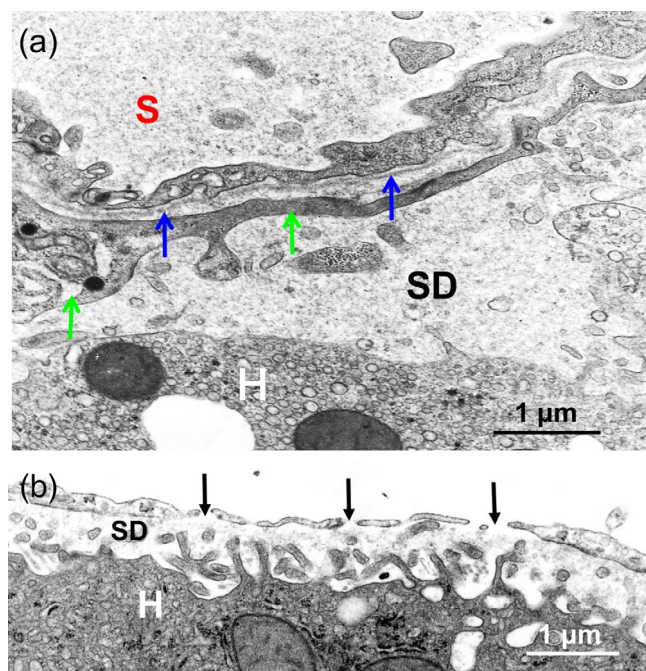


FIGURE 3 (a) TEM micrograph illustrates capillarization of the sinusoid in a baboon liver with advanced fibrosis after chronic alcohol consumption. The endothelial cell cytoplasm is thickened and is completely defenestrated. A basal lamina (blue arrows) is seen beneath the endothelium. The space of Disse is greatly widened to as much as 3 μm . The hepatocyte (H) is denuded of microvilli. An attenuated process of an activated hepatic stellate cell (green arrows) is visible in juxtaposition with the basal lamina, presumably providing support to the basal lamina and forming an addition barrier to the passage of materials across the Disse's space. (b) A normal fenestrated endothelium (arrows) is included for comparison. The endothelium is thin and fenestrated and lacks a basal lamina. Note hepatocyte (H) microvilli projecting into the space of Disse. S, sinusoid lumen; SD, space of Disse. (a) Mak and Mei (2017), with permission of Wiley

cause a perturbation in hepatic microcirculation and compromise the bidirectional exchange of materials across the space of Disse between the sinusoidal blood and parenchymal cells, which leads to hepatocellular dysfunction (Martinez-Hernandez & Martinez, 1991).

Capillarization of sinusoids is not specific to the type of liver injury, for it can occur in chronic liver disease of various causes such as alcoholic liver fibrosis (Horn et al., 1985; Urashima et al., 1993), primary biliary cirrhosis (Babbs et al., 1990), and autoimmune hepatitis (Xu et al., 2003). The structural alterations have been found in experimental models of hepatic fibrosis that used dimethylnitrosamine (Jézéquel et al., 1987; Ryhanen et al., 1996), CCl_4 (Martinez-Hernandez & Martinez, 1991; Nakayama et al., 1991), selenium (Dubuisson et al., 1995), and serum porcine (Bhunchet & Fujieda, 1993). Because sinusoidal capillarization in alcoholic liver disease with fibrosis has been most

studied previously, this area will be selected for review here, first in humans and then various animals chronologically. To that end, for each published study, the findings, implications, and conclusions are reviewed and highlighted.

4 | ALCOHOLIC LIVER DISEASE (HUMANS)

4.1 | Capillarization of sinusoids in alcoholic cirrhosis

Schaffner and Popper (1963) were the first to describe the histogenesis of capillarization of sinusoids in diseased human livers. Liver biopsies of patients with pathologies ranging from acute hepatitis to chronic hepatitis and to alcoholic cirrhosis were examined by TEM. In hepatitis samples, ultrastructural changes start with thickening of the sinusoidal endothelial cells with signs of loss of endothelial fenestrations—then called stomata. The space of Disse is widened from a normal width of $\sim 0.5 \mu\text{m}$ to several μm wide and contains abundant collagenous fibrils and proteinaceous extracellular matrix. In these sample livers, a distinct basement membrane is lacking beneath the sinusoidal endothelial cells. The hepatocyte surface facing the space of Disse in the affected area has shortened microvilli or few microvilli. In the livers of alcoholic patients with advanced long-standing and active cirrhosis, the endothelial lining is totally devoid of fenestrations and mostly surrounded by a continuous basement membrane, with some discontinuous segments. The space of Disse is further widened by collagenous matrix. These tripartite structural changes result in transformation of the sinusoids into systemic vascular capillaries, a process termed capillarization of sinusoids. The capillarized sinusoidal endothelium changes an open circulation system to closed circulation between the plasma and hepatocytes, which may aggravate preexisting hepatocellular insufficiency.

4.2 | Defenestration and capillarization of sinusoids in acinar zone 3 of early alcoholic fibrosis before cirrhosis

Alcohol consumption characteristically causes fibrotic changes in acinar zone 3 (centrilobular zone), which consists of perivenous fibrosis and perisinusoidal/pericellular fibrosis. Horn et al. (1985, 1987) studied liver needle biopsies of alcoholics and nonalcoholics without cirrhosis by TEM in conjunction with SEM. SEM analysis of normal human liver showed fenestration size is smaller, but the frequency of fenestrations is greater in zone 3, compared to zone 1. Consequently, the fenestration porosity of zone

3 was higher than that of zone 1, which is in line with the data reported earlier in rats (Wisse et al., 1983). In biopsy specimens of 15 alcoholics with or without fibrosis, a significantly lower number of fenestrations and porosity of the endothelium in zone 3 was observed in comparison to nonalcoholics. TEM examination of alcoholic liver with zone 3 fibrosis revealed the presence of a basal lamina underneath the endothelium, which was less fenestrated. In areas where basal laminae are present, sieve plate formation in endothelial cells was absent, with only single fenestrations noted. The space of Disse was widened and contained abundant collagenous fibrils in proximity to hepatic stellate cells that showed active fibrogenesis, which could cooperate, in part, with endothelial cells in the formation of basal lamina. Notwithstanding, the data did not reveal whether basal lamina formation precedes the defenestration process or vice versa. These changes reflect that the sinusoids undergo the capillarization process early on in alcoholic liver injury before the histogenesis of advanced fibrosis stages. This pathologic event in alcoholics is in sharp contrast to sinusoidal capillarization in other chronic liver disease that largely occurs in end stage cirrhosis. It is worth noting that basal lamina formation also occurs in alcoholics without zone 3 fibrosis, but not in a nonalcoholic liver. The on-going process of sinusoidal capillarization in early fibrosis stage may provoke liver injury in chronic alcoholics.

4.3 | Capillarization of sinusoids in alcoholic liver disease occurs at early liver fibrosis stage and progresses to end stage cirrhosis

Liver biopsies of alcoholic patients and nonalcoholic patients with mild, moderate, severe fibrosis and end stage cirrhosis were studied by TEM (Urashima et al., 1993). Capillarization of sinusoids is represented by the defenestration of endothelial cells and formation of basement membrane, which occurs with mild fibrosis early in alcoholic liver disease, but not in a non-alcoholic liver. The number of cases showing sinusoidal capillarization increases as fibrosis progresses to cirrhosis. In the stage of severe fibrosis and cirrhosis, no significant difference in the number of cases was found between alcoholic and nonalcoholic disease. Factor VIII-related antigen (FVIII-Ag) expression was used as a marker for transformation of sinusoids into systemic type capillaries (Babbs et al., 1990; Mak et al., 2014). Based on the extent of FVIII-Ag immunoreactivity in the liver lobules, the incidence of sinusoidal transformation correlates with sinusoidal capillarization. However, not all sinusoids in the liver lobules were capillarized and some remained sinusoidal.

4.4 | Markers of basement membrane: Collagen type IV and laminin expression in hepatic fibrosis of alcoholics

Collagen type IV and laminin are two principal basement membrane-associated proteins (Mak & Mei, 2017). Their codistribution in the space of Disse reflects the formation of perisinusoidal basement membrane. In normal liver, collagen IV immunoreactivity is observed along the sinusoidal lining, but laminin immunoreactivity is absent, which could explain the lack of a morphologically identifiable basement membrane underlying the endothelial cells (Hahn et al., 1980; Mak & Mei, 2017). In alcoholic patients, immunoreactive laminin becomes detectable and colocalized with collagen IV in the centrilobular area around the hepatic central vein, but not in the periportal area (Tsutsumi et al., 1993). Moreover, expression of collagen IV and laminin is higher in the early stage of alcoholic fibrosis than in the later stages, and the expression is greater than that in the corresponding types of nonalcoholic liver disease. Collagen IV and laminin codistribution expression indicates perisinusoidal basement membrane formation commences at an early stage of alcoholic liver fibrosis, in accordance with the TEM observation in early alcoholic liver injury (Horn et al., 1985; Urashima et al., 1993). Significantly, because basement membrane is an integral component of the capillarized sinusoid, its appearance *a priori* signals capillarization of the hepatic sinusoid.

The question is raised whether alcohol consumption provokes defenestration and basement membrane formation at the fatty liver stage before the onset of fibrosis. This is discussed as follows.

4.5 | Absence of sinusoidal structural changes in alcoholic steatosis

Percutaneous liver biopsies from six alcoholic patients with greater than 12 months of drinking (60 g/day) and control patients without liver disease were studied (Sztark et al., 1986). All alcoholics had steatosis of varying degrees but no evidence of perisinusoidal fibrosis. TEM in conjunction with morphometric analysis revealed no structural alterations of the sinusoidal endothelial cells and the space of Disse in either acinar zone 3 or zone 1. These findings indicate that heavy alcohol consumption does not alter the sinusoidal structure in a liver with steatosis in the absence of fibrosis.

Thus far, the aforementioned investigations on the sinusoidal endothelium in alcoholic liver disease were

descriptive. There were speculations about the link of the histopathology to impairment of material exchanges between the sinusoid blood and liver parenchyma and provocation of hepatic dysfunction. It was demonstrated earlier in rats that the porosity of the fenestrated sieve plate controls the passage of chylomicrons and their remnants from the sinusoidal circulation into the space of Disse (Fraser et al., 1978; Naito & Wisse, 1978). Chronic exposure to ethanol reduces the porosity of the endothelium and alcoholics manifest hyperlipidemia. This scenario led to the hypothesis that the low porosity associated with defenestration may contribute to the hyperlipoproteinemia observed in some people who drink heavily—review below.

4.6 | Defenestration and alcohol type V hyperlipoproteinemia in alcoholics

Clark et al. (1988) tested the hypothesis in a 33-year-old man with a 10-year history of heavy alcohol consumption. On light microscopy, the needle biopsy of the liver exhibited only slight patchy steatosis and a moderate increase in perisinusoidal fibrosis without cirrhosis. TEM showed collagen and some basement membrane material in the space of Disse. SEM revealed that fenestrations were sparse in the endothelial cells but were not quantified by number or size. These structural changes are in accordance with a low porosity of the sieve plate that presents a barrier to the usual passage of chylomicron remnants to hepatocytes for lipid metabolism. This results in reduced hepatic clearance of chylomicron remnants from the circulation and subsequent elevated blood lipoprotein levels. The patient had type V hyperlipoproteinemia, with chylomicron and very low-density lipoprotein (VLDL) triglycerides of 55 mmol/L and cholesterol of 16 mmol/L in the $d < 1.006$ kg/L ultracentrifugation fraction as well as an elevated cholesterol and high-density lipoprotein fraction (2.2 mmol/L). Incidentally, in alcohol-induced type V hyperlipoproteinemia, abstinence from alcohol substantially reduces the triglyceride levels, which suggests that the liver sieve has regained its normal porosity and function (Fraser et al., 1995).

Chronic alcohol consumption in baboons elicits a spectrum of hepatic lesions observed in alcoholics, namely fatty liver, fibrosis, and cirrhosis (Lieber & DeCarli, 1974). Since perisinusoidal fibrosis of the Disse's space is associated with defenestration and capillarization of sinusoids, it is expected that alcohol ingestion has an impact on the sinusoidal endothelium in baboons—as in alcoholic humans. This topic is reviewed as follows.

5 | SUBHUMAN PRIMATE BABOONS

There were only three studies related to structures of the sinusoidal endothelium in baboons—one on aging, one on induced-diabetes, and a third one on alcohol consumption. Aged baboons demonstrate pseudocapillarization, which is a less severe form of sinusoidal capillarization (Cogger et al., 2003). Diabetes in baboons provokes the loss of endothelial fenestrations and a reduction in the porosity (Jamieson et al., 2007), which mirrors the alterations seen in alcoholic baboons—see below.

5.1 | Chronic alcohol consumption in baboons with hepatic fibrosis: Defenestration and reduced porosity before cirrhosis

Sixteen adolescent baboons (*Papio hamadryas*) were paired nutritionally adequate diets that contained 50% of total calories as ethanol or isocaloric carbohydrate for a period of 4–112 months (Mak & Lieber, 1984). The alcohol-fed baboons all had steatosis, and all but one developed perivenular fibrosis, perisinusoidal/pericellular fibrosis, and septa formation. Based on the duration of alcohol consumption and the severity of the fibrosis, the baboons were grouped into two (4–24 months and 61–112 months) and compared with their pair-fed controls that showed normal histology at the time of the study. By SEM, the number of fenestrations decreased by 58% in baboons fed alcohol for 4–24 months, and the loss persisted in baboons fed alcohol for 61–112 months (Figure 4). Concurrently, the fenestration diameter increased by 40% after 4–24 months of alcohol feeding and by 19% after 61–112 months of feeding. The enlargement of fenestrations is potentially related to an attempt to compensate for the diminished fenestrated surface due to obliteration of some fenestrations. Yet, this is not sufficient alone to explain the loss, because the sinusoidal porosity in baboons fed alcohol for 4–24 months and 61–112 months amounted to 84 and 58% of their respective controls. Thus, the decreased porosity correlates not only with the duration of alcohol consumption but also the severity of hepatic fibrosis. In this baboon study, no assessment of the incidence of sinusoidal capillarization was performed. Notwithstanding, it is significant to note that the change in endothelial porosity is accompanied by fibrosis of the Disse's space with enhanced transformation of fibrogenic hepatic stellate cells into transitional myofibroblast-like cells, which is correlated with the greater depletion of hepatic vitamin A content (Mak et al., 1984) (Figure 4). Significantly, the changes in

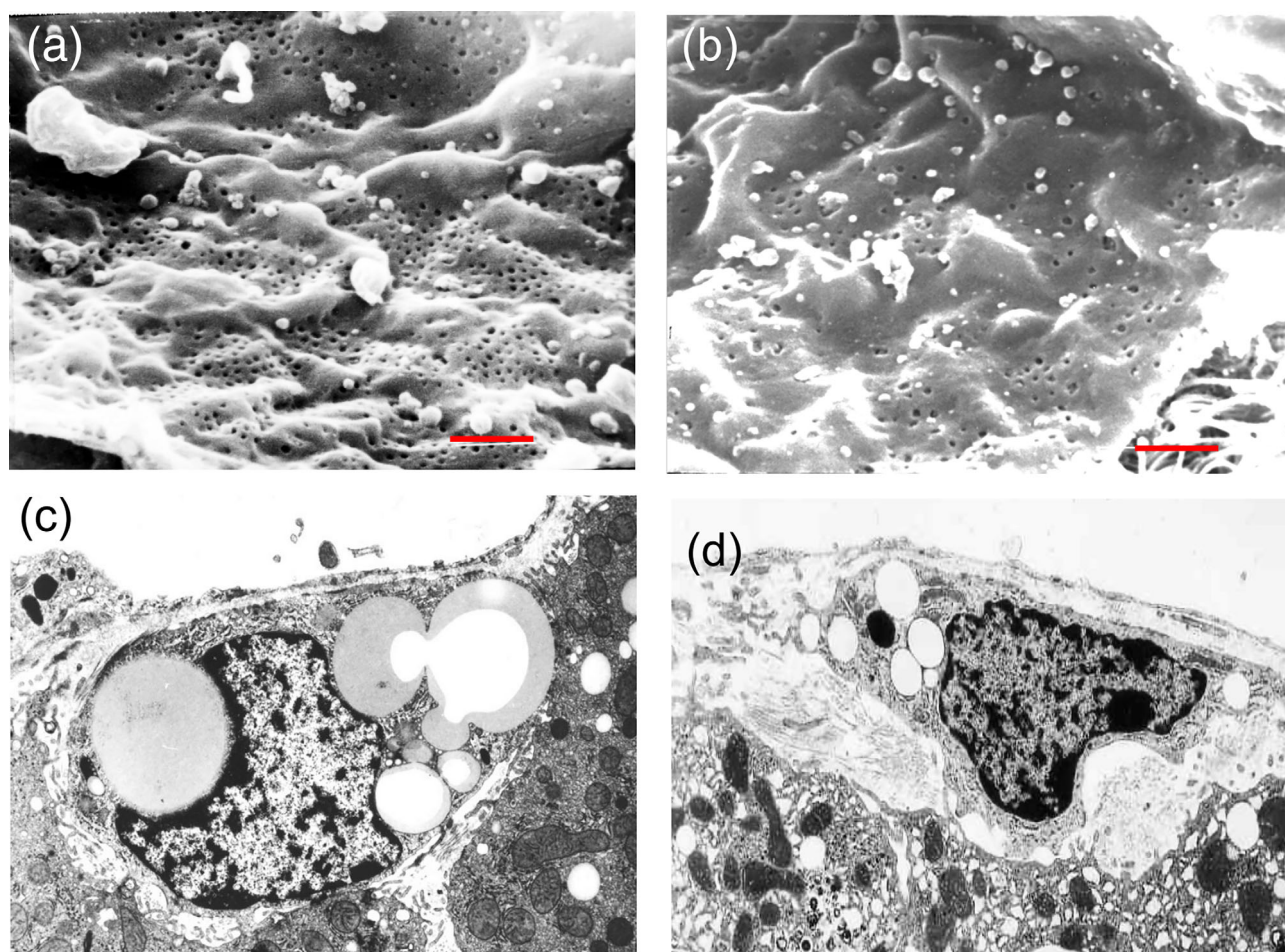


FIGURE 4 (a, b) SEM micrographs of sinusoidal endothelium. (a) Normal baboon liver. The endothelium shows fenestrations arranged in sieve plates. In comparison, the image in (b) is from a baboon chronically fed alcohol for 104 months that had developed advanced stage of septal fibrosis. Organized sieve plates are not recognized and the fenestrations are scattered in the endothelium. Scale bar = 1 μ m. (c, d) TEM micrographs of hepatic stellate cells (HSCs). (c) Quiescent HSC from a normal baboon. The HSC resides in the space of Disse and displays voluminous lipid droplets—containing vitamin A—that obscure the cell organelles. Few strands of fibrils are seen in the matrix around the cell. Original magnification, 4,000 $\times$. (d) Activated HSC from an alcoholic baboon. The lipid droplets are smaller and the RER is more apparent. The Disse's space contains an increased amount of collagen fibrils, producing perisinusoidal fibrosis. Original magnification, 7,000 $\times$. (c) Mak et al. (1984), with permission of Elsevier

alcoholic baboons with hepatic fibrosis before the onset of cirrhosis have been reproduced in alcoholic humans (Horn et al., 1985, 1987).

Having reviewed the alcohol-associated alterations of the sinusoids in alcoholic humans and alcoholic baboons, we now examine the impacts of alcohol in experimental rodent model of alcohol consumption—in following sections.

6 | CHRONIC ETHANOL INGESTION IN RATS AND MICE

Here we present the published studies in chronological order taking into consideration the variations in the formulation of the ethanol diets, route of administration, duration

of feeding, and methods of assessment that together may account for the outcomes of the investigations.

6.1 | Ethanol in water: 28 days ingestion

Rats were given a 33% mixture of 95% ethanol in water (vol/vol) via the esophagus in the morning and at night for 28 days (Fraser, Bowler, & Day, 1980). Control rats received 50% sucrose in water. Two hours after the last dose of ethanol, livers were perfusion fixed with chylomicrons added to the perfusate. By TEM, the diameter of sinusoidal fenestrations was enlarged by 20% (from 100 to 120 nm). However, the number, porosity, and their zonal distribution were not assessed. On TEM images, large

chylomicrons and small chylomicrons (remnants, defined below) were seen in the sinusoidal lumen of control rats, while small particles were present in the space of Disse (Figure 5). In ethanol-dosed rats, large chylomicrons also appear in the space of Disse, presumably having passed through the enlarged fenestrations into the space of Disse. In physiological terms, increasing the accessibility of the large chylomicrons to hepatocytes may be a factor in the pathogenesis of alcoholic fatty liver.

6.2 | Ethanol in sucrose solution and ethanol withdrawal: 3 months feeding

Rats were chronically fed ethanol (35%, wt/vol) containing 12% sucrose for 3 months (Kanaoka et al., 1988) and the fenestration morphology was assessed by SEM. The ethanol diet decreased the fenestration number while the fenestration diameter was increased in zone 3, opposing the values of the control rats. These changes resulted in a 50% higher endothelial porosity, unlike the lower porosity

demonstrated in human alcoholics and in alcoholic baboons (Horn et al., 1987; Mak & Lieber, 1984). Withdrawal of ethanol was found to normalize the porosity to the control values. Acute ethanol administration had no effect on the number and size of fenestrations, which contrasts with the findings of Charels et al. (1986) and Fraser et al. (1982) discussed in section 7.

6.3 | Lieber–DeCarli ethanol-containing liquid diet: 8 weeks feeding

Rats were fed (Lieber & DeCarli, 1982, 1994) liquid diets containing ethanol (5%) or isocaloric carbohydrate for controls (Tanikawa et al., 1991). Ethanol feeding for 8 weeks decreased the number of fenestrations and increased the fenestration diameter, which was associated with a reduction in endothelial porosity in both the centrilobular and periportal sinusoids. The decreased porosities contrast the data reported by Kanaoka et al. (1988) above but these changes are in accord with those found in human alcoholics and in alcohol-fed baboons (Horn et al., 1987; Mak & Lieber, 1984). Interestingly, TEM showed there was marked endocytic activity associated with the sinusoidal endothelial cells after chronic ethanol consumption, which could be a factor leading to the reduced porosity.

6.4 | Lieber–DeCarli ethanol-containing liquid diet: 6 weeks feeding

Rats were fed ethanol (Lieber & DeCarli, 1982, 1994) or control liquid diets (Okanoue et al., 1999), as in section 6.3. After 6 weeks of ethanol feeding, the endothelial porosity was higher in zone 3 in association with fewer fenestrations and an increased diameter of fenestrations. The findings are like those reported in rats by Kanaoka et al. (1988), but the higher porosity data are dissimilar to the lower porosity data of Tanikawa et al. (1991). The sinusoids in zone 3 appear to be tortuous and narrow, which is thought to be related to the ballooning of hepatocytes compressing the sinusoids. Therefore, the higher sinusoidal porosity in zone 3 could compensate for the hypoxia and hypertension brought about by the structural change of the sinusoids occurring in this zone.

6.5 | Disruption of sieve plates and formation of gaps: 14 weeks feeding

Rats were chronically fed ethanol with a complete liquid diet (14 weeks) or sucrose-containing drinking water as

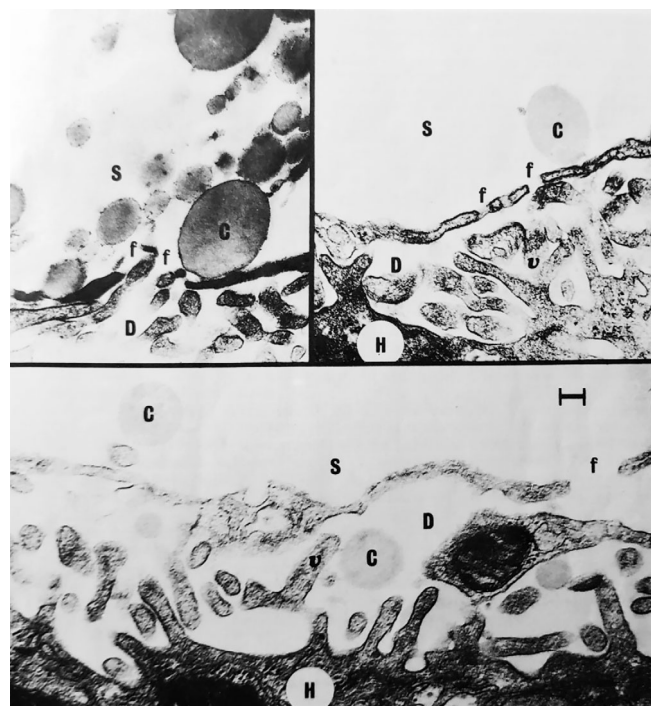


FIGURE 5 The upper micrographs are taken from normal rat live sinusoids. The endothelial lining separates the lumen (S) from the space of Disse (D). Small fenestrations (f) are marked. Large chylomicrons (C) are seen in the sinusoidal lumen. Microvilli of hepatocytes are present in the space of Disse. The lower micrograph is from ethanol-dose rat showing a large fenestration (f) and a large chylomicron (C) within the space of Disse. v, microvilli; H, hepatocytes. Original magnification, 44,000 $\times$. Scale bar: 100 nm. Fraser, Bowler, Day, Dobbs, et al. (1980), with permission of Elsevier

controls (12.5 weeks) (Sarphe et al., 1996). To rule out possible artifact induced by fixation procedure, livers were fixed by immersion (no perfusion), immersion preceded by perfusion, and by perfusion with glutaraldehyde. Regardless of the modes of administration or the fixation procedures, alcohol feeding caused a disruption of the sieve plate with the appearance of gaps, $\sim 1\text{--}2\ \mu\text{m}$ in size. In pathophysiology terms, the presence of gaps potentially allows freer communication between sinusoids and hepatocytes due to the loss of selective filtration of chylomicrons and their derivatives. The formation of gaps has been ascribed to preparation of liver samples for microscopy under high perfusion pressure (Fraser, Bowler, Day, Dobbs, et al., 1980; Wisse et al., 1984). Moreover, such large gaps have not been found in other studies with rats fed ethanol for a shorter or a comparable duration.

6.6 | Capillarization of sinusoids and basement membrane markers: 9 weeks feeding

Ethanol at 5 g/kg bw was administered by intragastric intubation to rats, 5 times per week (Xu et al., 2004). The ethanol solution was mixed with pyrazole, corn oil, carbonyl iron, xanthan gum, and maltose. After 9 weeks, the hepatocytes showed fatty change, swollen mitochondria, and necrosis. Hepatic stellate cells appeared activated and there were inflammatory cell infiltrates. TEM revealed a loss of endothelial fenestrations, appearance of an underlying basal lamina, and some degree of perisinusoidal fibrosis. Immunoreactive collagen IV and laminin were present in the perisinusoidal area, compatible with basement membrane formation and capillarization of the sinusoids. However, no zonal distribution of defenestration or porosity was reported.

6.7 | Capillarization of sinusoids and refenestration after ethanol withdrawal: 4–12 weeks feeding

Rats were given ethanol solution at increasing doses of 8–10 g/kg bw 3 times a day for 4, 8, and 12 weeks by intragastric intubation (Wang et al., 2005). Under TEM, loss of endothelial fenestrations was observed starting at 4 weeks and the defenestration became more significant at 8 and 12 weeks. The discontinuous endothelial basal lamina was apparent beginning at 8 weeks and the complete basal lamina was visible at 12 weeks. The time course of structural alterations is consistent with the notion that defenestration precedes the appearance of

basement membrane formation. However, no zonal distribution of basement membrane formation or changes in the porosity was reported. Interestingly, 12 weeks after the withdrawal of ethanol, the endothelium appeared refenestrated, and the incidence of basement membrane formation diminished.

6.8 | Loss of fenestrations with disorganization of sieve plates in mice: 26 days feeding

Mice were chronically fed an ethanol-containing liquid diet (4%, wt/vol) (Sarphe et al., 1997). Ethanol feeding diminished the number of fenestrations at 26 days. The normal sieve plate pattern became disorganized with the remaining fenestrations uniformly distributed in the endothelial cells. TEM showed the presence of collagenous fibrils in the space of Disse, but no sign of perisinusoidal basement membrane formation. With longer ethanol feeding at 56 days, there was further reduction in the frequency of fenestrations. Endothelial cell porosity was not determined in this study. The response of mice to chronic ethanol differed markedly from what was described in rats fed a similar diet (Sarphe et al., 1996). Yet the reduction in fenestration number in the mice is like other rat studies as well as what is seen in alcoholics and alcohol-fed baboons (Clark et al., 1988; Horn et al., 1985, 1987; Mak & Lieber, 1984; Urashima et al., 1993).

7 | ACUTE ETHANOL INGESTION IN RATS, MICE, AND RABBITS

7.1 | Enlargement of fenestrations in rats: 60 min exposure

Thirty-three percent of ethanol in water was given by stomach intubation to rats (1 mL/100 g bw) (Fraser et al., 1982) and rats were subsequently killed 1 h after the ingestion. Livers were perfusion-fixed under constant volume or constant pressure for SEM evaluation. The diameters of 500–1,000 random fenestrations from each rat were measured. There was a significant 24% increase in the diameter of the fenestrations under constant volume fixation and a 33% increase under constant pressure fixation.

7.2 | Increase in diameter and number of fenestrations in rats: 60 min exposure

A more detailed quantitative assessment of sinusoidal fenestrations by SEM was presented in which rats were

administered 30% and 40% of ethanol in drinking water by esophageal intubation and killed 60 min thereafter (Charels et al., 1986). The 40% ethanol-treated rats had a blood alcohol concentration two times as high as what was found in the 30% ethanol group. Acute ethanol dose increased the diameter of periportal fenestrations (35%), whereas the number decreased (−48%), which resulted in a lower overall porosity (−4.5%). Centrilobular fenestrations changed with comparable values (+43, −56, and −8.6%, respectively). It appears that ethanol has a stronger effect on diminishing the centrilobular porosity than the periportal porosity. The combination of increased fenestration diameter and decreased fenestration number could be explained by a coalescence process of fenestrations.

7.3 | Enlargement of fenestrations in rats and effect of methylpyrazole: 30 min exposure

Rats were given ethanol gastric intubation at 0.5, 1.0, and 2 g/kg bw and were killed 30 min after the intubation (Mori et al., 1991). Assessed by SEM, the diameter of endothelial fenestrations in both acinar zone 1 and zone 3 was enlarged with increasing dose of ethanol, reaching nearly 20% greater as compared to controls. The effect is like the findings of Fraser et al. (1982) and Charels et al. (1986). However, no fenestration number or porosity data were available. The fenestrations showed no significant changes in the acute ethanol-treated rats given methylpyrazole, a competitive inhibitor of alcohol dehydrogenase (ADH) (Blomstrand et al., 1979), which implicates involvement of ethanol oxidation generating acetaldehyde and acetate in the elicitation of fenestration enlargement—the thought is consistent with the data from *in vitro* LSEC cell culture, discussed see section 8.2 below.

7.4 | Decrease in fenestration number and diameter in rats: 60 min exposure

Ethanol was infused at 8 mg/mL via the mesenteric branch/portal vein into rats (Takashimizu et al., 1999). Blood flow was found to be decreased and portal pressure was elevated at 10 min after the infusion, demonstrating portal hypertension. Blood ethanol concentration reached 1.77 g/mL at 60 min (equivalent to ~36 mM, which is a normal value). In the ethanol-infused rats, the diameter and number of fenestrations in zone 1 were both decreased (40 and 40%, respectively). Similarly, the decrease in fenestration diameter and number both

occurred in zone 3 (27 and 37%, respectively). The decrease in fenestration diameter contrasted with the data of Charels et al. (1986). Porosities were not measured. These findings implicate a link between sinusoidal defenestration and portal hypertension caused by ethanol. This study also demonstrates an involvement of endothelin-1 in hepatic circulatory disturbance in acute ethanol administration.

7.5 | Decreased fenestration diameter in rabbits: 10 min exposure

In this study, New Zealand male white rabbits were administered a single dose of ethanol intravenously (0.75 g/kg) (Jacobs et al., 2009). Ethanol blood concentration peaked at 1.1 g/L (~23 mM) 10 min after the injection. The diameter of endothelial fenestrations was determined by TEM rather than by SEM. Ethanol-dosed rabbits demonstrated a slight 7% decrease in the fenestration diameter compared to controls (96 vs. 103 nm). The effect is considered the earliest morphological alteration in the liver induced by ethanol. Incidentally, the control value of fenestration diameter (103 nm) in these rabbits was much higher than that previously reported in New Zealand female rabbits (average 49 nm) using SEM (Wright et al., 1983). The latter authors also observed that the rabbit fenestration size was about half of the rat fenestration.

This early effect of ethanol on the fenestration diameter in rabbits is slight (7%) compared to the 38% decrease in rats reported in the study of Takashimizu et al. (1999) albeit at comparable blood ethanol levels. The rabbit data are also not in accord with the enlargement of fenestrations observed in rats by others (Charels et al., 1986; Fraser, Bowler, Day, Dobbs, et al., 1980; Mori et al., 1991). The data discrepancy is presumably related to differences in species, methods of measurements with TEM versus SEM, blood ethanol levels and timing as well—10 versus 30–60 min.

7.6 | Gap formation in mice: 3-hr treatment

Acute ip administration of ethanol (3 g/kg bw) was found to induce the appearance of gaps coexisting with normal sieve plate fenestrations in mice sinusoidal endothelial cells (Sarphie et al., 1997). The effect was transient. Thus, acute ethanol treatment in mice elicits endothelial gaps that replace the normal fenestrations, which is like that seen in rats that chronically consume ethanol for 14 weeks (Sarphie et al., 1996).

8 | IN VITRO EFFECTS OF ETHANOL IN LSEC CULTURE

8.1 | Enlargement of fenestrations: 90 min exposure

Isolated rat sinusoidal endothelial cells were maintained in culture for 17 hr (Charels et al., 1986) before ethanol solution (95%) at 2 g/L was added to the culture over 90 min. This ethanol dose is equivalent to 43 mM, a level generally seen in the blood after ethanol administration and commonly used in in vitro studies. Ethanol treatment enlarged the endothelial fenestrations by 20% (251 vs. 208 nm in control) like the acute in vivo effect of ethanol observed in rats (Charels et al., 1986; Fraser et al., 1982; Mori et al., 1991), which suggests that ethanol may have a direct action on the fenestration structure.

8.2 | Dynamics of fenestration-associated cytoskeleton in enlargement of fenestrations: 90 min exposure

Isolated rat sinusoidal endothelial cells were maintained in culture for 8 hr and then treated with 2 g/L of ethanol 99.8% (vol/vol) for 90 min (Braet et al., 1995, 1996). Ethanol was found to increase the diameter of endothelial cell fenestrae by 5–10%. It has been shown that ethanol and its metabolite acetaldehyde have a direct interaction with microtubules and filaments of hepatocytes (Matsuda et al., 1979, 1983). Therefore, the effect of ethanol on the size of fenestrations likely involves dynamic changes of the cytoskeletal microtubules surrounding the sieve plates as well as actin and myosin forming rings around individual fenestrations (Braet et al., 1995, 1998; Cogger et al., 2010).

8.3 | Absence of effect on fenestration size: 3 min exposure

Isolated rat sinusoidal cells were cultured for 6 hr and ethanol (100, 200, and 400 mg/dL) was added to the culture and incubated for another 30 min (Mori et al., 1991). This ethanol treatment was not found to affect the size of fenestrations. This data deviated from the findings of Charels et al. (1986) and Braet et al. (1995, 1996), which may be due to use of lower ethanol concentrations of 5 times or more.

8.4 | General comments on LSEC culture

In these three aforementioned studies, the zonal origin of isolated LSECs was unknown, given the

heterogeneity of endothelial cells across the liver lobule of rats and humans (Mak & Shin, 2021; Sorensen & Smedsrod, 2020; Strauss et al., 2017; Xie et al., 2010). Likewise, the viability, purity, and identity were not reported. While the use of LSEC culture may offer a tool to assess direct actions of ethanol on fenestration dynamics, there are technical issues concerning isolated LSECs in cell culture (DeLeve & Maretti-Mira, 2017). LSECs lose their fenestrations within hours of isolation and culture, which limits the duration of experiments that can be validly performed (Elvevold et al., 2008; Lalor et al., 2006; Poisson et al., 2017). The degree of cell viability is very dependent upon culture conditions, such as the presence of growth factors (esp. vascular endothelial growth factor) and the degree of oxygenation (Martinez et al., 2008; Sorensen & Smedsrod, 2020). Above all, the question whether LSEC culture expresses ADH or cytochrome P-450E1 (CYP2E1) that catalyzes the oxidation of ethanol and produces acetaldehyde, acetate, and other toxic metabolites (Lieber, 1997) must be addressed.

9 | ETHANOL AND HEPATOTOXIC DRUGS COMBINED

9.1 | Synergistic effects of ethanol and cocaine on sinusoids in mice and rats

The study was conducted to assess the synergistic effects of ethanol and cocaine on hepatic sinusoids (McCuskey et al., 1993). Mice and rats were fed a modified Lieber and DeCarli (1982, 1994) liquid diet of either dextrin-maltose (control) or ethanol that amounted to 27% (mice) and 36% (rats) of the overall calories for up to 15 weeks. Cocaine was injected to animals daily ip. In mice, ethanol and cocaine combined elicited severe sinusoidal damage showing thickening of the sinusoidal endothelium, loss of endothelial fenestrations, collagenization of the Disse's space, and deposition of perisinusoidal basement membrane. These changes are compatible with sinusoidal capillarization. Also, parenchymal necrosis and reduced centrilobular sinusoidal blood flow were observed. Such changes were seen to a much lesser extent in animals treated with either cocaine or ethanol alone, implicating synergism of ethanol and cocaine. In contrast to the mice, rats showed only scant fibrosis and minor alterations of the sinusoidal endothelial cells with ethanol and cocaine. Therefore, there appears to be differences in species sensitivity to ethanol and cocaine toxicity. The synergistic toxic effects of combined ethanol and cocaine raise concern about severe liver damage by alcohol abuse in cocaine addicts.

9.2 | Ethanol binge drinking exacerbates acetaminophen (APAP) cytotoxicity in mice

Mice were subjected to a single, weekend-type ethanol binge (4 g/kg bw every 12 hr $\times$ 5 doses or three-weekly binges (McCuskey et al., 2005). Such binges alone did not produce injury to the sinusoidal endothelium. But ethanol binging was found to enhance the sinusoidal endothelial injury provoked by oral gavage of APAP in the mice. The injury was marked by the development of numerous gaps in the endothelial cells, possibly by coalescence of endothelial fenestrations. This sinusoidal injury occurred prior to signs of parenchymal cell injury. Depletion of intracellular GSH levels concomitantly with induction of CYP2E1 by ethanol is implicated in the APAP hepatotoxicity. There is no clear data that indicates CYP2E1 is induced in the endothelial cells, whereas the CYP2E1 has been shown to be highly inducible in Kupffer cells of rats after chronic ethanol feeding (Koivisto et al., 1996).

9.3 | Ethanol feeding and lipopolysaccharide in rats

Rats were fed a nutrient adequate liquid diet containing either alcohol or dextrin (control) for 14 weeks (Sarphie et al., 1995). Livers from alcohol-fed rats demonstrated a massive loss of endothelial sieve plate integrity with replacement of gaps exceeding 1 μ m in diameter. Bacterial LPS administration to alcohol-fed rats accentuated the alcohol-induced sinusoidal structural alterations. The effect is attributed to LPS direct action on Kupffer cells that in turn secrete mediators affecting the structure and function of sinusoidal endothelial cells (Deaciuc & Spitzer, 1996).

10 | SUMMARY AND COMMENTS ON DYNAMIC CHANGES OF ENDOTHELIAL FENESTRATIONS AND INCIDENCE OF SINUSOIDAL CAPILLARIZATION

Table 1 is a survey of the dynamic changes of fenestration and incidence of capillarization of sinusoids in alcoholic liver disease, chronic and acute ethanol ingestion, in vitro LSEC culture, and ethanol+drug/toxin. Sinusoidal capillarization is associated with hepatic fibrosis in humans and in rats. There is a high concordance in the incidence of decreased fenestration number and increased fenestration diameter in alcoholic liver fibrosis and chronic alcohol feeding of baboons and rats. The number of cases reporting endothelial porosity is not sufficiently represented; however, when there

is morphologic evidence of capillarization of sinusoids, the porosity is a priori abolished or at the very least significantly diminished. In the case of alcoholic liver with fibrosis in humans and in baboons, a lower porosity correlates with fewer fenestrations and increased fenestration diameter. In chronic ethanol-fed rats, a higher porosity is associated with decreased fenestration number and increased fenestration diameter, which is opposite of that seen in humans and baboons. A coalescence process of fenestrations could explain the combination of a decrease in fenestration number and an increase in fenestration diameter. In the short term, the ethanol ingestion dilates fenestrations in rats and contracts the fenestrations in rabbits, but there is insufficient data on its effects on the fenestration number or porosity. In LSEC culture, ethanol provokes fenestration enlargement, an effect like that observed in rats after acute ethanol ingestion and chronic ethanol feeding. In general, the differences in the anatomy of fenestrations—fenestration dynamics—do not appear to be related to etiology and species.

Some of the discrepancies of data in animal feeding studies could be related to variations of ethanol concentrations in the diets, nutrient composition of the diets, routes of ethanol administration, duration of alcohol feeding, and lacking use of pair-fed controls—with the exception of the baboon study (Mak & Lieber, 1984) and the rat studies by Sarphie et al. (1995, 1996, 1997). In executing ethanol feeding protocols, pair-fed controls are critical in equalizing caloric intake between ethanol-fed and control animals (Lieber & DeCarli, 1982, 1994). In addition, variations in specimen preparations for microscopy, the number of fenestrations analyzed, and statistical methods could have led to authors' published conclusions on the fenestration dynamics in response to dietary ethanol treatment. Going forward, at a minimum the following information should be provided: methods used to measure fenestrations, values for both the number and diameter that are required for calculating the porosity, and zonal distribution of porosity considering the heterogeneity of fenestrations along the liver lobule—centrilobular versus periportal (Wisse et al., 1983).

11 | ETHANOL, SIEVE PLATE, HYPERLIPIDEMIA, AND ATHEROSCLEROSIS

The notion that the fenestrated sieve plate plays a regulatory role in sieving chylomicrons and chylomicron remnants from the sinusoidal circulation into the space of Disse and subsequent postprandial hyperlipidemia that may impact systemic vascular disease in association with alcoholism was examined. Postprandial hyperlipidemia and chylomicron remnants are atherogenic (Fraser et al., 2012; Zilversmit, 1979).

TABLE 1 Alcohol-associated modulation of fenestration dynamics and capillarization of sinusoids

Studies	Fenestration number	Fenestration diameter	Fenestration porosity	Gaps	Cap. of sinusoids	Fibrosis	BM marker
Alcoholic liver dis.							
Schaffner and Popper (1963)	Decrease	*	*	*	Yes	Yes	*
Horn et al. (1985, 1987)	Decrease	Increase	Decrease	*	Yes	Yes	*
Sziark et al. (1986)	No effect	No effect	No effect	*	n	Steatosis	*
Clark et al. (1988)	Decrease	*	*	*	Yes	Yes	*
Urashima et al. (1993)	Decrease	*	*	*	Yes	Yes	Yes
Hahn et al. (1980)	*	*	*	*	*	Yes	Yes
Tsutsumi et al. (1993)	*	*	*	*	*	Yes	Yes
Chronic ethanol feeding							
Mak and Lieber (1984), baboons	Decrease	Increase	Decrease	*	*	Yes	*
Fraser, Bowler, Day, Dobbs, et al. (1980), rats	*	Increase	*	*	*	n	*
Kanaoka et al. (1988), rats	Decrease	Increase	Increase	*	n	n	*
Tanikawa et al. (1991), rats	Decrease	Increase	Decrease	*	n	n	*
Okanoue et al. (1999), rats	Decrease	Increase	Increase	*	n	n	*
Xu et al. (2004), rats	Decrease	*	*	*	n	Yes	Yes
Wang et al. (2005), rats	Decrease	*	*	*	Yes	Yes	*
Sarphie et al. (1996), rats	*	*	*	Yes	n	n	*
Sarphie et al. (1997), mice	Decrease	*	Decrease	*	n	n	*
Acute ethanol ingestion							
Fraser et al. (1982), rats	*	Increase	*	*	n	n	*
Charels et al. (1986), rats	Decrease	Increase	Decrease	*	n	n	*
Kanaoka et al. (1988), rats	No effect	No effect	No effect	*	n	n	*
Mori et al. (1991), rats	*	Increase	*	*	n	n	*
Takashimizu et al. (1999), rats	Decrease	Decrease	*	*	n	n	*
Jacobs et al. (2009), rabbits	*	Decrease	*	*	n	n	*
Sarphie et al. (1997), mice	*	*	*	Yes	n	n	*
LSEC Culture							
Charels et al. (1986)	*	Increase	*	*	n	n	*
Mori et al. (1991)	*	No effect	*	*	n	n	*
Braet et al. (1995, 1996)	*	Increase	*	*	n	n	*

(Continues)

TABLE 1 (Continued)

Studies	Fenestration number	Fenestration diameter	Fenestration porosity	Gaps	Cap. of sinusoids	Fibrosis	BM marker
Ethanol+drug/toxin							
McCuskey et al. (1993), ethanol+cocaine, mice ^a	Decrease	*	*	*	Yes	Yes	*
McCuskey et al. (2005), ethanol binge+APAP, mice	*	*	*	Yes	n	n	*
Sarphie et al. (1995), ethanol+LPS, rats	*	*	*	Yes	n	n	*

Note: Asterisks indicate no data or not determined.

Abbreviations: Cap, capillarization; BM marker, perisinusoidal basement membrane, collagen IV/laminin; n, negative result (no capillarization or fibrosis).

^aNo corresponding changes in rats.

11.1 | Fenestration size and porosity influence the passage of chylomicrons and chylomicron remnants

Wisse (1970) postulated that the sieve plate containing fenestrations filters chylomicrons and other lipoproteins according to size. Chylomicrons (also known as ultra-low-density lipoproteins) are triglyceride-rich lipoprotein particles formed in the intestinal enterocytes from dietary fats. The diameter of chylomicrons is 100–1,000 nm, depending on the dietary fat load, and they are too large to pass through the endothelial fenestrations that are 50–150 nm in diameter (Fraser et al., 2012). Chylomicrons are metabolized to chylomicron remnants by lipoprotein lipase present on systemic vascular endothelial cells. The remnant particles contain apolipoprotein (apoE and apoB-48) and are triglyceride-depleted, but rich in cholesteryl ester and fat-soluble vitamins. Compared with their parent chylomicrons, the remnants are 30–80 nm, 3–10 times smaller particles. As such, the remnants can pass through the fenestrations into the space of Disse, and subsequently are cleared from the circulation. Then chylomicron remnants bind to hepatocyte membrane receptors (low-density lipoprotein receptor and lipoprotein-receptor related protein) that recognize apoE for uptake into hepatocytes and subsequent metabolism (Fraser et al., 2012). It has been shown that there is a close correlation between the diameter of fenestrations and chylomicron remnants presence in the Disse's space. TEM revealed that chylomicrons larger than the fenestrations are present only in sinusoids while the smaller remnants are seen also in the space of Disse (Naito & Wisse, 1978; Fraser et al., 1978). In corroboration, radio-labeled chylomicrons of different sizes are trapped differentially by the liver, such that smaller particles are trapped to a greater extent than those larger than 100 nm. Similar results indicating hepatic uptake of liposomes and colloidal gold particles based on size have been reported (Hardonk et al., 1985; Romero et al., 1999).

Consequently, defenestration—reduction in fenestration diameter or number—and associated low porosity of the sinusoidal endothelium impedes the passage of chylomicron remnants into the space of Disse, thereby diminishing their hepatic clearance from the circulation after meals. This contributes to postprandial hyperlipidemia, because remnants are still relatively rich in triglycerides (Fraser et al., 1995, 2012) and are atherogenic as well. This phenomenon manifests in people who drink heavily, which provides a connection between alcohol abuse and hyperlipidemia, discussed as follows.

11.2 | Links between ethanol, defenestration, and hyperlipoproteinemia

Long-term alcohol consumption leads to the pathogenesis of capillarization of sinusoids before the onset of cirrhosis, as elaborated above. However, the endothelial cells may defenestrate even before the sinusoids become capillarized with acquisition of basement membrane. In this scenario, induction of defenestration along with reduced porosity could be a mechanism that underlies the hyperlipoproteinemia manifested in chronic alcoholics, as hypothesized by Clark et al. (1988). The defenestrated endothelium acts as a barrier for chylomicron remnants filtered through the sieve plate to reach the receptors on hepatocyte surface. This leads to enhancement of hepatic synthesis of triglyceride-rich lipoproteins due to a lack of downregulation of 3-hydroxy-3-methylglutaryl CoA reductase and, consequently, elevated levels of lipoproteins in the circulation. Incidentally, in alcohol-induced type V hyperlipoproteinemia, abstinence from alcohol substantially reduces the plasma triglyceride levels suggesting refenestration of the sinusoids and regaining a normal porosity (Fraser et al., 1995). Indeed, in rats, the withdrawal of ethanol following a long-term feeding regimen led to restoration of the fenestrations and the porosity to control values (Kanaoka et al., 1988; Wang et al., 2005). On the other hand, acute ethanol was found to dilate the fenestrations, at least transiently (Charels et al., 1986; Fraser et al., 1982; Mori et al., 1991). The enlargement of fenestrations is thought to enhance hepatic clearance of dietary lipids from the circulation, which could account, at least in part, for the beneficial effects of low to moderate doses alcohol consumption against cardiovascular risks (Brinton, 2010; Fernández-Solà, 2015).

12 | CONCLUDING REMARKS AND FUTURE PERSPECTIVE

The groundbreaking discovery of capillarization of sinusoids in alcoholic cirrhosis was published 59 years ago to date (Schaffner & Popper, 1963), and the original concept of the “liver sieve” was developed by Wisse and Fraser in the 1970s. Defenestration of the sinusoidal endothelium in alcohol-fed baboons with hepatic fibrosis before the development of cirrhosis was reported in 1984. Since then, there have been a number of investigations of alcoholic liver fibrosis in humans that corroborate the impact of alcohol consumption on sinusoidal defenestration and capillarization. At the same time, findings in animals after chronic ethanol feeding and short-term ethanol exposure have further provided experimental evidence that ethanol modulates dynamic changes of endothelial

fenestrations. Fenestrations largely form sieve plates that allow passage of circulating chylomicron remnants into the space of Disse and subsequent uptake by hepatocytes for lipid metabolism. A correlation between alcohol consumption, defenestration, and hyperlipoproteinemia was noted in an alcoholic. Accordingly, it was hypothesized that the loss of the liver sieve integrity contributes to alcohol-associated hyperlipoproteinemia. The deficiency in sieving capacity impairs hepatic clearance of chylomicron remnants and accounts for postprandial hyperlipoproteinemia, which is atherogenic favoring pathogenesis of systemic vascular disease, including atherosclerosis. The histopathogenesis of capillarized sinusoids in alcoholic liver disease and the modulation of fenestration parameters by ethanol thus far are descriptive and lack mechanisms of action. Alcohol consumption in conjunction with antagonists or agonists that modify the toxicity of ethanol could reveal the pathophysiology actions of ethanol on sinusoidal capillarization. LSEC culture may be a tool to provide clues to the mechanisms of action of ethanol and its metabolite acetaldehyde in modulating the dynamics of fenestration-associated cytoskeleton. This also raises the question of whether LSECs have the ADH and/or CYP2E1 to oxidize ethanol to acetaldehyde, oxyradicals and acetate. Going forward, use of CYP2E1 knockouts may aid in affirming the involvement of ethanol in this process. It will be of great interest to examine whether ethanol mediates the dynamics of fenestrations via a paracrine signaling mechanism. Further, utilization of the NIAAA-preferred 10-day chronic and binge ethanol feeding model (Bertoli et al., 2013), which synergistically induces liver injury, inflammation, and fatty liver mimicking acute-on-chronic alcoholic liver injury in patients, or the zebrafish models of alcoholic steatosis (Schneider et al., 2017) may yield new insight into the actions of ethanol on fenestration dynamics. Understanding the molecules, biochemistry, and physiology mediating the morphogenesis and maintenance of sinusoidal fenestrations could lead to the development of pharmacological interventions to prevent defenestration or to facilitate refenestration of endothelial cells, thereby offering strategies for managing systemic vascular disease in alcoholism.

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REFERENCES

- Babbs, C., Haboubi, N. Y., Mellor, J. M., Smith, A., Rowan, B. P., & Warnes, T. W. (1990). Endothelial cell transformation in primary biliary cirrhosis: A morphological and biochemical study. *Hepatology*, 11, 723–729.

- Bertoli, A., Mathews, S., Ki, S. H., Wang, H., & Gao, B. (2013). Mouse model of chronic and binge ethanol feeding (the NIAAA model). *Nature Protocols*, 8, 627–637.
- Bhunchet, E., & Fujieda, K. (1993). Capillarization and venularization of hepatic sinusoids in porcine serum-induced rat liver fibrosis: A mechanism to maintain liver blood flow. *Hepatology*, 18, 1450–1458.
- Blomstrand, R., Ostling-Wintzell, H., Löf, A., McMartin, K., Tolf, B. R., & Hedström, K. G. (1979). Pyrazoles as inhibitors of alcohol oxidation and as important tools in alcohol research: An approach to therapy against methanol poisoning. *Proceedings of the National Academy of Sciences of the United States of America*, 76, 3499–3503.
- Braet, F. (2004). How molecular microscopy revealed new insights into the dynamics of hepatic endothelial fenestrae in the past decade. *Liver International*, 24, 532–539.
- Braet, F., De Zanger, R., Baekeland, M., Crabbé, E., Van Der Smissen, P., & Wisse, E. (1995). Structure and dynamics of the fenestrae-associated cytoskeleton of rat liver sinusoidal endothelial cells. *Hepatology*, 21, 180–189.
- Braet, F., Kalle, W. H., De Zanger, R. B., De Grooth, B. G., Raap, A. K., Tanke, H. J., & Wisse, E. (1996). Comparative atomic force and scanning electron microscopy: An investigation on fenestrated endothelial cells in vitro. *Journal of Microscopy*, 181, 10–17.
- Braet, F., Spector, I., De Zanger, R., & Wisse, E. (1998). A novel structure involved in the formation of liver endothelial cell fenestrae revealed by using the actin inhibitor misakinolide. *Proceedings of the National Academy of Sciences of the United States of America*, 95, 13635–13640.
- Braet, F., & Wisse, E. (2002). Structural and functional aspects of liver sinusoidal endothelial cell fenestrae: A review. *Comparative Hepatology*, 1, 1.
- Braet, F., Wisse, E., Bomans, P., Frederik, P., Geerts, W., Koster, A., Soon, L., & Ringer, S. (2007). Contribution of high-resolution correlative imaging techniques in the study of the liver sieve in three-dimensions. *Microscopy Research and Technique*, 70, 230–242.
- Brinton, E. A. (2010). Effects of ethanol intake on lipoproteins and atherosclerosis. *Current Opinion in Lipidology*, 21, 346–351.
- Charels, K., De Zanger, R. B., Van Bossuyt, H., Van Der Smissen, P., & Wisse, E. (1986). Influence of acute alcohol administration on endothelial fenestrae of rat livers: An in vivo and in vitro scanning electron microscopic study. In A. Kirn, D. L. Knook, & E. Wisse (Eds.), *Cells of the hepatic sinusoid* (Vol. 1, pp. 497–502). Rijswijk: Kupffer Cell Foundation.
- Clark, S. A., Bramwell Cook, H., Oxner, R. B. G., Angus, H., George, P., & Fraser, R. (1988). Defenestration of hepatic sinusoids as a cause of hyperlipoproteinaemia in alcoholics. *Lancet*, 332, 1225–1227.
- Cogger, V. C., Hunt, N. J., & Le Couteur, D. G. (2020). Fenestrations in the liver sinusoidal endothelial cells. In I. M. Arias, H. J. Atler, J. L. Boyer, D. E. Cohen, D. A. Shafritz, S. S. Thorgeirsson, & A. W. Wolkoff (Eds.), *The liver: Biology and pathobiology* (Vol. 35, 6th ed., pp. 435–443). Hoboken, NJ: John Wiley & Sons, Ltd.
- Cogger, V. C., McNerney, G. P., Nyunt, T., DeLeve, L. D., McCourt, P., Smedsrød, B., Le Couteur, D. G., & Huser, T. R. (2010). Three-dimensional structured illumination microscopy of liver sinusoidal endothelial cell fenestrations. *Journal of Structural Biology*, 171, 382–388.
- Cogger, V. C., Warren, A., Fraser, R., Ngu, M., McLean, A. J., & Le Couteur, D. G. (2003). Hepatic sinusoidal pseudocapillarization with aging in the non-human primate. *Experimental Gerontology*, 38, 1101–1107.
- Deaciuc, I. V., & Spitzer, J. J. (1996). Hepatic sinusoidal endothelial cell in alcoholemia and endotoxemia. *Alcoholism, Clinical and Experimental Research*, 20, 607–614.
- DeLeve, L. D., & Maretti-Mira, A. C. (2017). Liver sinusoidal endothelial cell: An update. *Seminars in Liver Disease*, 37, 377–387.
- Dubuisson, L., Boussarie, L., Bedin, C. A., Balabaud, C., & Bioulac-Sage, P. (1995). Transformation of sinusoids into capillaries in a rat model of selenium-induced nodular regenerative hyperplasia: An immunolight and immunoelectron microscopic study. *Hepatology*, 21, 805–814.
- Elvevold, K., Smedsrød, B., & Martinez, I. (2008). The liver sinusoidal endothelial cell: A cell type of controversial and confusing identity. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 294, G391–G400.
- Fernández-Solà, J. (2015). Cardiovascular risks and benefits of moderate and heavy alcohol consumption. *Nature Reviews. Cardiology*, 12, 576–587.
- Fraser, R., Bosanquet, A. G., & Day, W. A. (1978). Filtration of chylomicrons by the liver may influence cholesterol metabolism and atherosclerosis. *Atherosclerosis*, 29, 113–123.
- Fraser, R., Bowler, L. M., & Day, W. A. (1980). Damage of rat liver sinusoidal endothelium by ethanol. *Pathology*, 12, 371–376.
- Fraser, R., Bowler, L. M., Day, W. A., Dobbs, B., Johnson, H. D., & Lee, D. (1980). High perfusion pressure damages the sieving ability of sinusoidal endothelium in rat livers. *British Journal of Experimental Pathology*, 61, 222–228.
- Fraser, R., Bowler, L. M., De Zanger, R. B., & Wisse, E. (1982). Agents related to fibrosis, such as alcohol and carbon tetrachloride, acutely affect endothelial fenestrae which may cause fatty liver. In U. Gerlach, G. Pott, J. Rauterberg, & B. Voss (Eds.), *Connective tissue of the normal and fibrotic human liver* (pp. 159–160). Stuttgart: Georg Thieme Verlag Thieme-Stratton Inc..
- Fraser, R., Cogger, V. C., Dobbs, B., Jamieson, H., Warren, A., Hilmer, S. N., & Le Couteur, D. G. (2012). The liver sieve and atherosclerosis. *Pathology*, 44, 181–186.
- Fraser, R., Dobbs, B. R., & Rogers, G. W. (1995). Lipoproteins and the liver sieve: The role of the fenestrated sinusoidal endothelium in lipoprotein metabolism, atherosclerosis, and cirrhosis. *Hepatology*, 21, 863–874.
- Hahn, E., Wick, G., Pencev, D., & Timpl, R. (1980). Distribution of basement membrane proteins in normal and fibrotic human liver: Collagen type IV, laminin and fibronectin. *Gut*, 21, 63–71.
- Hardonk, M. J., Harms, G., & Koudstaal, J. (1985). Zonal heterogeneity of rat hepatocytes in the in vivo uptake of 17 nm colloidal gold granules. *Histochemistry*, 83, 473–477.
- Horn, T., Christoffersen, P., & Henriksen, J. H. (1987). Alcoholic liver injury: Defenestration in noncirrhotic livers—A scanning electron microscopic study. *Hepatology*, 7, 77–82.
- Horn, T., Junge, J., & Christoffersen, P. (1985). Early alcoholic liver injury: Changes of the Disse space in acinar zone 3. *Liver*, 5, 301–310.
- Jacobs, F., Wisse, E., & De Geest, B. (2009). Early effect of a single intravenous injection of ethanol on hepatic sinusoidal endothelial fenestrae in rabbits. *Comparative Hepatology*, 8, 4.

- Jamieson, H. A., Cogger, V. C., Twigg, S. M., McLennan, S. V., Warren, A., Cheluvappa, R., Hilmer, S. N., Fraser, R., de Cabo, R., & Le Couteur, R. G. (2007). Alterations in liver sinusoidal endothelium in a baboon model of type 1 diabetes. *Diabetologia*, 50, 1969–1976.
- Jézéquel, A. M., Mancini, R., Rinaldesi, M. L., Macarri, G., Venturini, C., & Orlandi, F. (1987). A morphological study of the early stages of hepatic fibrosis induced by low doses of dimethylnitrosamine in the rat. *Journal of Hepatology*, 5, 174–181.
- Kanaoka, H., Okanou, T., Sawa, Y., Kachi, K., Ohta, Y., Morimoto, M., Kagawa, K., Yuki, T., & Takino, T. (1988). The effects of ethanol on the sinusoidal endothelial fenestration of rat liver. *Kanzo (The Japan Society of Hepatology)*, 29, 198–206.
- Koivisto, T., Mishin, V. M., Mak, K. M., Cohen, P. A., & Lieber, C. S. (1996). Induction of cytochrome P-4502E1 by ethanol in rat Kupffer cells. *Alcoholism, Clinical and Experimental Research*, 20, 207–212.
- Lalor, P. F., Lai, W. K., Curbishley, S. M., Shetty, S., & Adams, D. H. (2006). Human hepatic sinusoidal endothelial cells can be distinguished by expression of phenotypic markers related to their specialised functions in vivo. *World Journal of Gastroenterology*, 12, 5429–5439.
- Lieber, C. S. (1997). Cytochrome P-4502E1: Its physiological and pathological role. *Physiological Reviews*, 77, 517–544.
- Lieber, C. S., & DeCarli, L. M. (1974). An experimental model of alcohol feeding and liver injury in the baboon. *Journal of Medical Primatology*, 3, 155–163.
- Lieber, C. S., & DeCarli, L. M. (1982). The feeding of alcohol in liquid diets: Two decades of applications and 1982 update. *Alcoholism, Clinical and Experimental Research*, 6, 523–531.
- Lieber, C. S., & DeCarli, L. M. (1994). Animal models of chronic ethanol toxicity. *Methods in Enzymology*, 233, 585–594.
- Mak, K. M., Leo, M. A., & Lieber, C. S. (1984). Alcoholic liver injury in baboons: Transformation of lipocytes to transitional cells. *Gastroenterology*, 87, 188–200.
- Mak, K. M., & Lieber, C. S. (1984). Alterations in endothelial fenestrations in liver sinusoids of baboons fed alcohol: A scanning electron microscopic study. *Hepatology*, 4, 386–391.
- Mak, K. M., & Mei, R. (2017). Basement membrane type IV collagen and laminin: An overview of their, basement membrane type IV collagen and laminin: An overview of their biology and value as fibrosis biomarkers of liver disease biology and value as fibrosis biomarkers of liver disease. *Anatomical Record*, 300, 1371–1390.
- Mak, K. M., Sehgal, P., & Harris, C. K. (2014). Factor VIII-related antigen detects phenotypic change of sinusoidal to vascular endothelium in hepatic fibrosis of elderly cadavers. *International Scholarly Research Notices*, 2014, 839560.
- Mak, K. M., & Shin, D. W. (2021). Hepatic sinusoids versus central veins: Structures, markers, angiocrines, and roles in liver regeneration and homeostasis. *Anatomical Record*, 304, 1661–1691.
- Martinez, I., Nedredal, G. I., Øie, C. I., Warren, A., Johansen, O., Le Couteur, D. G., & Smedsrød, B. (2008). The influence of oxygen tension on the structure and function of isolated liver sinusoidal endothelial cells. *Comparative Hepatology*, 7, 4.
- Martinez-Hernandez, A., & Martinez, J. (1991). The role of capillarization in hepatic failure: Studies in carbon tetrachloride-induced cirrhosis. *Hepatology*, 14, 864–874.
- Matsuda, Y., Baraona, E., Salaspuuro, M., & Lieber, C. S. (1979). Effects of ethanol on liver microtubules and Golgi apparatus. Possible role in altered hepatic secretion of plasma proteins. *Laboratory Investigation*, 41, 455–463.
- Matsuda, Y., Takada, A., Kanayama, R., & Takase, S. (1983). Changes of hepatic microtubules and secretory proteins in human alcoholic liver disease. *Pharmacology, Biochemistry, and Behavior*, 18, 479–482.
- McCuskey, R. S., Bethea, N. W., Wong, J., McCuskey, M. K., Abril, E. R., Wang, X., Ito, Y., & DeLeve, L. D. (2005). Ethanol bingeing exacerbates sinusoidal endothelial and parenchymal injury elicited by acetaminophen. *Journal of Hepatology*, 42, 371–377.
- McCuskey, R. S., Eguchi, H., Nishida, J., Krasovich, M. A., McDonnell, B., Jolley, C. S., Abril, E. R., Earnest, D. L., & Watson, R. R. (1993). Effects of ethanol and cocaine alone or in combination on the hepatic sinusoids of mice and rats. In E. Wisse & D. L. Knook (Eds.), *Cells of the hepatic sinusoid* (Vol. 4, pp. 376–380). Leiden: Kupffer Cell Foundation.
- Mönkemöller, V., Schüttelz, M., McCourt, P., Sørensen, K., Smedsrød, B., & Huser, T. (2014). Imaging fenestrations in liver sinusoidal endothelial cells by optical localization microscopy. *Physical Chemistry Chemical Physics*, 16, 12576–12581.
- Mori, T., Okanou, T., Sawa, Y., Itoh, Y., Kanaoka, H., Hori, N., Enjyo, F., Nakagawa, Y., Kagawa, K., & Kashima, K. (1991). Effect of ethanol on the sinusoidal endothelial fenestration of rat liver—In vivo and in vitro study. In E. Wisse, D.L. Knook & R.S. McCuskey (Eds.), *Cells of the hepatic sinusoid* (Vol. 3, pp. 469–471). Leiden, The Netherlands: Kupffer Cell Foundation.
- Naito, M., & Wisse, E. (1978). Filtration effect of endothelial fenestrations on chylomicron transport in neonatal rat liver sinusoids. *Cell and Tissue Research*, 190, 371–382.
- Nakayama, Y., Takahara, T., Miyabayashi, C. R., Itoh, H., Watanabe, A., Sasaki, H., Muragaki, Y., Ooshima, A., & Inoue, K. (1991). Ultrastructural localization of type IV collagen and laminin in the Disse space of rat liver with carbon tetrachloride induced fibrosis. *Liver*, 11, 260–271.
- O'Reilly, J. N., Cogger, V. C., Fraser, R., & Le Couteur, D. G. (2010). The effect of feeding and fasting on fenestrations in the liver sinusoidal endothelial cell. *Pathology*, 42, 255–258.
- Okanoue, T., Sakamoto, S., Mori, T., Sawa, Y., Kanaoka, H., Nishioji, K., & Itoh, Y. (1999). Role of sinusoidal endothelial cells in alcoholic liver disease. In K. Tanikawa & T. Ueno (Eds.), *Liver diseases and hepatic sinusoidal cells* (pp. 190–198). Springer.
- Poisson, J., Lemoine, S., Boulanger, C., Durand, F., Moreau, R., Valla, D., & Rautou, P. E. (2017). Liver sinusoidal endothelial cells: Physiology and role in liver diseases. *Journal of Hepatology*, 66, 212–227.
- Romero, E. L., Morilla, M. J., Regts, J., Koning, G. A., & Scherphof, G. L. (1999). On the mechanism of hepatic trans-endothelial passage of large liposomes. *FEBS Letters*, 448, 193–196.
- Ryhanen, L., Stenback, F., Ala-Kokko, L., & Savolainen, E. R. (1996). The effect of malotilate on type III and type IV collagen, laminin and fibronectin metabolism in dimethylnitrosamine-induced liver fibrosis in the rat. *Journal of Hepatology*, 24, 238–245.
- Sarphie, G., D'Souza, N. B., Van Thiel, D. H., Hill, D., McClain, C. J., & Deaciuc, I. V. (1997). Dose- and time-

- dependent effects of ethanol on functional and structural aspects of the liver sinusoid in the mouse. *Alcoholism, Clinical and Experimental Research*, 21, 1128–1136.
- Sarphie, T. G., D'Souza, N. B., Spitzer, J. J., & Deaciuc, I. V. (1996). Chronic alcohol feeding in liquid diet or in drinking water has similar effects on electron microscopic appearance of the hepatic sinusoid in the rat. *Alcoholism, Clinical and Experimental Research*, 20, 973–979.
- Sarphie, T. G., Deaciuc, I. V., Spitzer, J. J., & D'Souza, N. B. (1995). Liver sinusoid during chronic alcohol consumption in the rat: An electron microscopic study. *Alcoholism, Clinical and Experimental Research*, 19, 291–298.
- Schaffner, F., & Popper, H. (1963). Capillarization of hepatic sinusoids in man. *Gastroenterology*, 44, 239–242.
- Schneider, A. C. R., Gregorio, C., Uribe-Cruz, C., Guizzo, R., Malysz, T., Faccioni-Heuser, M. C., Longo, L., & de da Silveira, T. R. (2017). Chronic exposure to ethanol causes steatosis and inflammation in zebrafish liver. *World Journal of Hepatology*, 9, 418–426.
- Sorensen, K. K., & Smedsrod, B. (2020). The liver sinusoidal endothelial cell: Basic biology and pathobiology. In I. M. Arias, H. J. Atler, J. L. Boyer, D. E. Cohen, D. A. Shafritz, S. S. Thorgeirsson, & A. W. Wolkoff (Eds.), *The liver: Biology and pathology* (Vol. 34, 6th ed., pp. 422–434). Hoboken, NJ: John Wiley & Sons, Ltd.
- Strauss, O., Phillips, A., Ruggiero, K., Bartlett, A., & Dunbar, P. R. (2017). Immunofluorescence identifies distinct subsets of endothelial cells in the human liver. *Scientific Reports*, 7, 44356.
- Sztark, F., Latry, P., Quinton, A., Balabaud, C., & Bioulac-Sage, P. (1986). The sinusoidal barrier in alcoholic patients without liver fibrosis. A morphometric study. *Virchows Archives A, Pathological Anatomy and Histopathology*, 409, 385–393.
- Takashimizu, S., Watanabe, N., Nishizaki, Y., Kawazoe, K., & Matsuzaki, S. (1999). Mechanisms of hepatic microcirculatory disturbances induced by acute ethanol administration in rats, with special reference to alterations of sinusoidal endothelial fenestrae. *Alcoholism, Clinical and Experimental Research*, 23, 39s–46s.
- Tanikawa, K., Noguchi, K., & Sata, M. (1991). Ultrastructural features of Kupffer cells and endothelial cells in chronic ethanol fed-rats. In E. Wisse, D. L. Knook, & R. S. McCuskey (Eds.), *Cells of the hepatic sinusoid* (Vol. 3, pp. 445–448). Kupffer Cell Foundation.
- Tsutsumi, M., Urashima, S., Matsuda, Y., Takase, S., & Takada, A. (1993). Changes in type IV collagen content in livers of patients with alcoholic liver disease. *Hepatology*, 17, 820–827.
- Urashima, S., Tsutsumi, M., Nakase, K., Wang, J. S., & Takada, A. (1993). Studies on capillarization of the hepatic sinusoids in alcoholic liver disease. *Alcohol and Alcoholism. Supplement*, 1B, 77–84.
- Vidal-Vanaclocha, F., & Barberá-Guillem, E. (1985). Fenestration patterns in endothelial cells of rat liver sinusoids. *Journal of Ultrastructure Research*, 90, 115–123.
- Wang, B. Y., Ju, X. H., Fu, B. Y., Zhang, J., & Cao, Y. X. (2005). Effects of ethanol on liver sinusoidal endothelial cells-fenestrae of rats. *Hepatobiliary & Pancreatic Diseases International*, 4, 422–426.
- Wisse, E. (1970). An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoids. *Journal of Ultrastructure Research*, 31, 125–150.
- Wisse, E., Braet, F., Luo, D., Vermijlen, D., Eddouks, M., Konstandoulaki, M., Empsen, C., & De Zanger, R. B. (1999). Endothelial cells of the hepatic sinusoids: A review. In K. Tanikawa & T. Ueno (Eds.), *Liver diseases and hepatic sinusoidal cells* (pp. 17–53). Tokyo: Springer-Verlag.
- Wisse, E., De Wilde, A., & De Zanger, R. (1984). Perfusion fixation of human and rat liver tissue for light and electron microscopy: A review and assessment of existing methods with special emphasis on sinusoidal cells and microcirculation. In A. M. F. O'Hare (Ed.), *Science of biological tissue preparation* (pp. 31–38). Chicago, IL: SEM Inc.
- Wisse, E., De Zanger, R. B., Charels, K., Van Der Smissen, P., & McCuskey, R. S. (1985). The liver sieve: Considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. *Hepatology*, 5, 683–692.
- Wisse, E., De Zanger, R. B., Jacobs, R., & McCuskey, R. S. (1983). Scanning electron microscope observations on the structure of portal veins, sinusoids and central veins in rat liver. *Scanning Electron Microscopy*, 1983, 1441–1452.
- Wright, P. L., Smith, K. F., Day, W. A., & Fraser, R. (1983). Small liver fenestrae may explain the susceptibility of rabbits to atherosclerosis. *Atherosclerosis*, 1983(3), 344–348.
- Xie, G., Wang, L., Wang, X., Wang, L., & DeLeve, L. D. (2010). Isolation of periportal, midlobular, and centrilobular rat liver sinusoidal endothelial cells enables study of zonated drug toxicity. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 299, G1204–G1210.
- Xu, B., Broome, U., Uzunel, M., Nava, S., Ge, X., Kumagai-Braesch, M., Hultenby, K., Christensson, B., Ericzon, B. G., Holgersson, J., & Sumitran-Holgersson, S. (2003). Capillarization of hepatic sinusoid by liver endothelial cell-reactive autoantibodies in patients with cirrhosis and chronic hepatitis. *American Journal of Pathology*, 163, 1275–1289.
- Xu, G. F., Wang, X. Y., Ge, G. L., Li, P. T., Jia, X., Tian, D. L., Jiang, L. D., & Yang, J. X. (2004). Dynamic changes of capillarization and peri-sinusoid fibrosis in alcoholic liver diseases. *World Journal of Gastroenterology*, 10, 238–243.
- Zapotoczny, B., Szafranska, K., Kus, E., Braet, F., Wisse, E., Chlopicki, S., & Szymonski, M. (2019). Tracking fenestrae dynamics in liver murine liver sinusoidal endothelial cells. *Hepatology*, 69, 876–888.
- Zilvermit, D. B. (1979). Atherogenesis: A postprandial phenomenon. *Circulation*, 60, 473–485.

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